THE UNIVERSITY OF ALBERTA

THE METABOLISM OF RADIOACTIVE YTTRIUM IN DOGS AND GUINEA PIGS WITH ATTEMPTED LOCALIZATION IN THE GASTRIC MUCOSA

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES

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BY

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ABSTRACT

The metabolism of yttrium^{90} in dogs and guinea pigs was investigated. Yttrium^{90} solution was administered intravenously, as ionic yttrium and in combination with several chelates. The effect of carrier and of various chelate concentrations, and the influence of intramuscular histamine on the deposition of yttrium^{90} were studied in the stomach, spleen, liver, kidney, bone marrow and dry bone of dogs and guinea pigs. Neither histamine nor chelate concentration had any effect on the deposition of yttrium in any of these tissues. In both dogs and guinea pigs, when ionic yttrium was administered it tended to concentrate largely in the spleen and liver. In dogs, EDTA and HEDTA chelated with yttrium with added carrier produced the highest concentration of yttrium in the gastric mucosa. In guinea pigs, only HEDTA chelated with yttrium with added carrier produced significant amounts in the gastric mucosa. When carrier free yttrium^{90} was used, none of the chelates tested produced a high concentration in the gastric mucosa.

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Chapter 1 HISTORICAL REVIEW

In spite of all the new and highly advertised medications, the treatment of the peptic ulcer diathesis is still a challenging problem. In fact, no ideal remedy is yet available which will satisfactorily prevent all its manifold manifestations, although a constant search is being made for new methods of treatment. The etiology of this disease is still an enigma, and before considering treatment, it is perhaps better first to discuss some of the theories of the cause of peptic ulcer.

The first recorded observation of ulcerative lesions of the stomach and duodenum as a cause of digestive disturbances, dates back to the Greek physician and medical practitioner Galen, who lived in the second century. Little more was discovered about the stomach and duodenum until 1752 when Reamur showed that the stomach contained acid. Some 72 years later, in 1824, Prout discovered that it was muriatic acid. Abercrombie, in 1828, and Cruveilhier, in 1829, were the first to describe accurately the morbid anatomy and symptoms of gastroduodenal ulcerative disease. As early as 1772, John Hunter, in a report to the Royal Society, in an attempt to explain why the stomach did not digest itself, proposed the theory that living tissue possessed a vital principle which prevented its digestion. This vague concept remained the only available theory on the subject until 1856 when Bernard and Pavy concluded that "parts of animals possessed of the living principle when taken into the stomach" are very definitely affected "by the powers of that viscus." Bernard postulated that the

presence of a continuously renewed protective epithelium prevented digestion and that pepsin was not absorbed by living cells. Cruveilhier believed that gastritis and duodenitis precede ulceration, while the Viennese pathologist Rokitansky, in 1849, stressed haemorrhagic erosions as the etiology. Virchow, in 1853, suggested that obstruction of a nutrient artery by an embolus or thrombus produced an infarct, which was then destroyed by the digestive gastric juice. Aschoff proposed that the ulcer was due to trauma resulting from eating coarse, rough food. In 1874, Bottcher attempted to establish gastroduodenal ulceration on an infectious basis. Connheim, in 1880, proposed that hyperchlorhydria was the cause of the chronic duodenal ulcer. In 1911, W. T. Mayo proposed the theory that traction on the anterior duodenal wall produced an anaemic area, and expressed the belief that this area was vulnerable to peptic digestion because of impaired circulation.

It is now generally agreed that the duodenal ulcer is due to the corrosive action of excessive amounts of acid peptic juice. The peptic activity of pure gastric juice at pH 4.5 to 5 is negligible, while at pH 1 it has the capacity to digest and destroy any living tissue. The result of this digestion is a lesion which is anatomically indistinguishable from the duodenal ulcer. Dragstedt has shown that the fasting stomach of the duodenal ulcer patient secretes from three to ten times as much gastric juice as does the normal, and claims that this hypersecretion is of nervous origin. It is generally believed that for cure, achlorhydria is unnecessary, in most cases, and that treatment need only correct the secretory abnormality by reducing the volume and acidity of

gastric secretion in the interdigestive phase to normal levels.

With this multitude of theories of the cause of peptic ulceration, it is no wonder that the recommended treatments were equally as numerous. In 1828 Abercrombie recommended milk and farinaceous foods. Johnson in 1831 recommended chalk, soda and magnesia for "pain in the stomach due to acidity". In 1835 Cruveilhier stated that "stomach ulcer" was curable and recognized the importance of dietary hygiene in preventing its recurrence. Fox and Forster, in 1872, emphasized the importance of rest. With the exception of sedatives, antispasmodics and psychotherapy, the modern basic concepts of medical management of the peptic ulcer were established before 1880. In the latter part of the nineteenth century Kussmaul, Ewald, Dieulafoy and others introduced gastric lavage, astringent irrigations, antispasmodic drugs, gastric rest and antacids in the treatment of the peptic ulcer. Conservative management was the treatment of choice and recourse to surgery was only in the event of emergency.

Surgical treatment of gastric diseases did not really begin until 1881, when in February of that year, Billroth did a partial gastric ressection on a patient with gastric carcinoma. In September of the same year, Anton Woelfler, working in Billroth's clinic in Vienna, performed the first successful gastroenterostomy. This patient also had gastric cancer. Pean (1879) had earlier done a gastroenterostomy but his patient died. Rydygier performed the first gastroenterostomy for peptic ulceration in 1884. In 1886 Heineke and Mikulicz individually performed and described the operation of pyloroplasty. Thus within a

period of five years were described the three major types of gastric surgery that are still used today; namely, gastric ressection, gastroenterostomy and pyloroplasty. In the early days surgical treatment of peptic ulcer was a formidable procedure and the mortality from gastroenterostomy was said to be as high as 65%. However, surgical techniques improved and the period 1881 to 1915 may be described as the golden era of the gastric surgeons. During this time there evolved a multitude of operations which revealed the ingeniousness of the surgeons of that day. Many of these procedures were based upon unsound physiological principles and were probably more dependent upon the imaginations of the surgeons involved. Some of the great gastric surgeons of this era were Billroth, Heineke, Mikulicz, von Haber, Roux, Finsterer, von Eiselberg, Jaboulay, Hofmeister, Polya, and many others. It is paradoxical that many of these men advocated procedures which frequently resulted in complications as troublesome as the one they set out to cure. Courvosier, in 1883, proposed the posterior gastroenterostomy and, in 1885, von Haber introduced the posterior retrocolic anastomosis.

In all surgical procedures the incidence of infection with its resultant increase in morbidity and mortality was high, especially since gastric surgery was frequently for malignancy where gastric acidity is decreased or absent. Surgeons went to great lengths to avoid infection and operations were devised in which anastomoses were performed without opening the mucosa at the time of operation. McGraw, in 1891, used an elastic ligature which was designed to cause necrosis of the tissue encompassed by it and thus establish the opening between the stomach and jejunum. Boari applied caustic to the mucosa which resulted in a slough

at some later date. Many procedures failed because the stoma did not remain patent. It was thought that this difficulty could be avoided by the use of a splint which would keep the stoma open until healing occurred. For this purpose, Senn described the use of decalcified bone plates in 1888, Robinson suggested rawhide plates, von Baracz used turnip plates in 1892, and in the same year Murphy used a button. All of these have long since been abandoned. In the early part of the twentieth century medical management fell into disrepute and the gastroenterostomy was in vogue. Von Mickulicz was a staunch advocate of gastroenterostomy and Moynihan declared that "the treatment of chronic duodenal ulcer should always be surgical." Murphy (1914) suggested the wide excision of the ulcer and Balfour (1917) advocated cautery to the ulcer.

However, in 1925, Berg and Lewisohn of New York reported a twenty-year follow-up on the operation of gastroenterostomy, ulcer excision, cautery puncture and pyloroplasty, and found that only 50% were cured and at least 30% developed anastomotic ulcers or a recurrence at the original ulcer site. The findings of Bier, Payr and others in Europe were essentially the same. It is unfortunate that the surgeons of this era did not recognize earlier that initial improvement follows any surgery on the stomach and duodenum, Their only criterion for cure was the immediate prevention of ulcer recurrence and little thought was given to the long-term effect. In fact, many patients which had been operated upon would have been better had they been left alone.

Meanwhile Sippy in 1915 established the principle that neutralization of hydrochloric acid in the stomach was the essential factor in proper

medical management and introduced the now well-known Sippy regimen. Sippy was able to show that pyloric obstruction, haemorrhage and even perforation could be combatted successfully by well planned medical management. It was largely due to the success of the Sippy regimen, the chaotic state of surgical therapy and its high mortality and morbidity and the large number of surgical failures that led to the Sippy regimen becoming the therapy of choice for peptic ulcer in the second and third decades of this century, and surgical treatment was reserved only for complications.

In the early part of the twentieth century basic research in gastric physiology was neglected in favour of the development of new surgical techniques. Notable workers in gastric physiology were Pavlov, who demonstrated the gastric secretory response to psychic stimuli in dogs, Edkins (1906)who proposed the presence of the antral hormone gastrin, which explained the disastrous results of the antral exclusion operations, and Mann and Williamson (1928) who shunted the alkaline duodenal secretions to the terminal fifteen centimeters of the ileum in sixteen dogs, fourteen of which subsequently developed jejunal ulcers. In the early thirties Dragstedt began emphasizing the importance of hydrochloric acid in the genesis of peptic ulcer and suggested the term "acid ulcer." In 1943 he advocated vagotomy combined with a drainage procedure as the surgical treatment of choice. Dragstedt has recently reported that the ulcer recurrence rate for such a procedure is approximately nine percent. In 1940 Code and Varco[1] showed that the intramuscular injection of histamine in beeswax in experimental animals produced a profound gastric secretory response which invariably resulted in peptic ulcers.

Parallelling the development of medical and surgical treatment of the peptic ulcer diathesis was the development of radiation therapy. It was only one year after the discovery of X-rays by Wilhelm Roentgen (1896), that they were used in the diagnosis of peptic ulcer, (Walsh, 1897). In 1917, Bruegel[2] suggested the use of a therapeutic dose of X-rays to the stomach in the treatment of this disease. Bensaude[3] in 1925 showed a decrease in gastric acidity in six of eleven patients receiving X-ray therapy to the stomach; and Viviani,[4] in 1931, studied the effect of radiation on histamine induced gastric secretion, reporting a definite depression of gastric activity which was variable in both amount and duration. In 1932, Emery[5] noted a temporary achlorhydria in four patients after X-ray to the stomach. Irradiation of the pyloric area in the treatment of polycythemia vera had no significant effect on gastric secretion; however, tumoricidal doses caused necrosis and ulceration of the mucosa. Working with dogs, Portis and Ahrens (1924)[6]; Ivy, McCarthy and Orndorff (1924)[7]; Case and Boldyreff (1928)[8]; Snell and Bollman (1934)[9]; found a variable and temporary reduction in gastric secretion following irradiation. The dose of X-rays employed in these studies was too variable and and the data collected on gastric secretions and the course of the peptic ulcer was too meagre to warrant any valid conclusions. Beginning in 1938, Levin, Palmer, Kirsner,[10,11,12,13] et al, began a study of the long-term effects of radiation therapy on peptic ulcer. By 1956 they had collected data on 723 duodenal ulcer patients, which had been followed for five to eighteen years. The recurrence rate per hundred patient years of symptoms before treatment was 102 and after treatment only 17. Similarly, the reduction of haemorrhage by irradiation of the stomach, was from 4.3

to 1.1. There were no undesirable side effects and no apparent increase in malignancy, or leukemia.

In 1942 Jenkins and McGeorge[14] used radium in an intragastric balloon in the treatment of duodenal ulcer. Simon[15], in 1949, coated a thin rubber balloon with phosphorus^{32} and inserted it into the Heidenhain pouch of five dogs, following which there was a marked decrease in acid secretion, raning from anacidity in two to a maximum of ten percent of the pre-irradiation level in the other three. The longest period of observation after irradiation of any animal was 125 days. Douglas, et al[16], (1951), using phosphorus^{32} but with a different technique of applying it to a rubber bag irradiated the stomachs of seven dogs, after which all dogs showed a reduction of free hydrochloric acid and total acid. Brown[17], in 1959, reported on the use of cobalt^{60} teletherapy as a means of irradiation for benign peptic ulcer in 72 patients, of which 39 had been followed for more than 12 months. All 72 patients showed radiological evidence of healing of the ulcer in two to three months; 64 of the 72 remained continuously asymptomatic and three developed a recurrence of the ulcer. In 1960 Littman[18] electroplated 100 millicuries of ruthenium-rhodium^{106} onto a small brass cylinder which was then placed in the stomach. Of six patients so treated, there was no significant effect on acidity although gastritis was evident at biopsy. Three patients which received 600, 800 and 1000 rep respectively to the gastric mucosa, developed large gastric ulcers which healed so slowly they required partial gastrectomy. Even these patients showed only minimal and transitory depression of gastric acidity.

Thus in conclusion it is apparent that the ideal treatment for the duodenal ulcer patient has not yet been found. Many patients find that under medical therapy the diet restrictions are too severe; they cannot tolerate large quantities of milk, cream and antacids; the side effects of antispasmodics and sedatives are too prominent; or the ulcer provoking mechanism is too powerful to be controlled by any course of medical management. For them there is recourse to surgery with its considerable morbidity, mortality and the threat of recurrence. Irradiation of the stomach by external means is undesirable since it is impossible to irradiate just the stomach by present techniques: all adjacent tissues, spleen, liver, retroperitoneal tissue, skin, dorsal and lumbar spine, etc., receive a considerable radiation dose. Similarly, many ulcers recurr after what appears to be an adequate of radiation. Direct radiation of the gastric mucosa by the application of a radioisotope to a rubber balloon or similar device is still experimental and preliminary results are discouraging. In theory at least, the ideal way to irradiate the acid producing cells of the gastric mucosa would be by means of a radioisotope which would be selectively taken up by these cells, in a way similar to that in which the thyroid selectively concentrates iodine. By proper selection of isotope, (i.e., a pure beta emitter), only a few millimeters of surrounding tissue would be irradiated, thus minimizing the irradiation of organs and tissue adjacent to the stomach. In 1955 Dudley and Greenberg[19] found that under certain circumstances yttrium^{90} could be selectively deposited in the gastric mucosa, and the following is a brief description of this metal, its chemistry and chelation.

REFERENCES

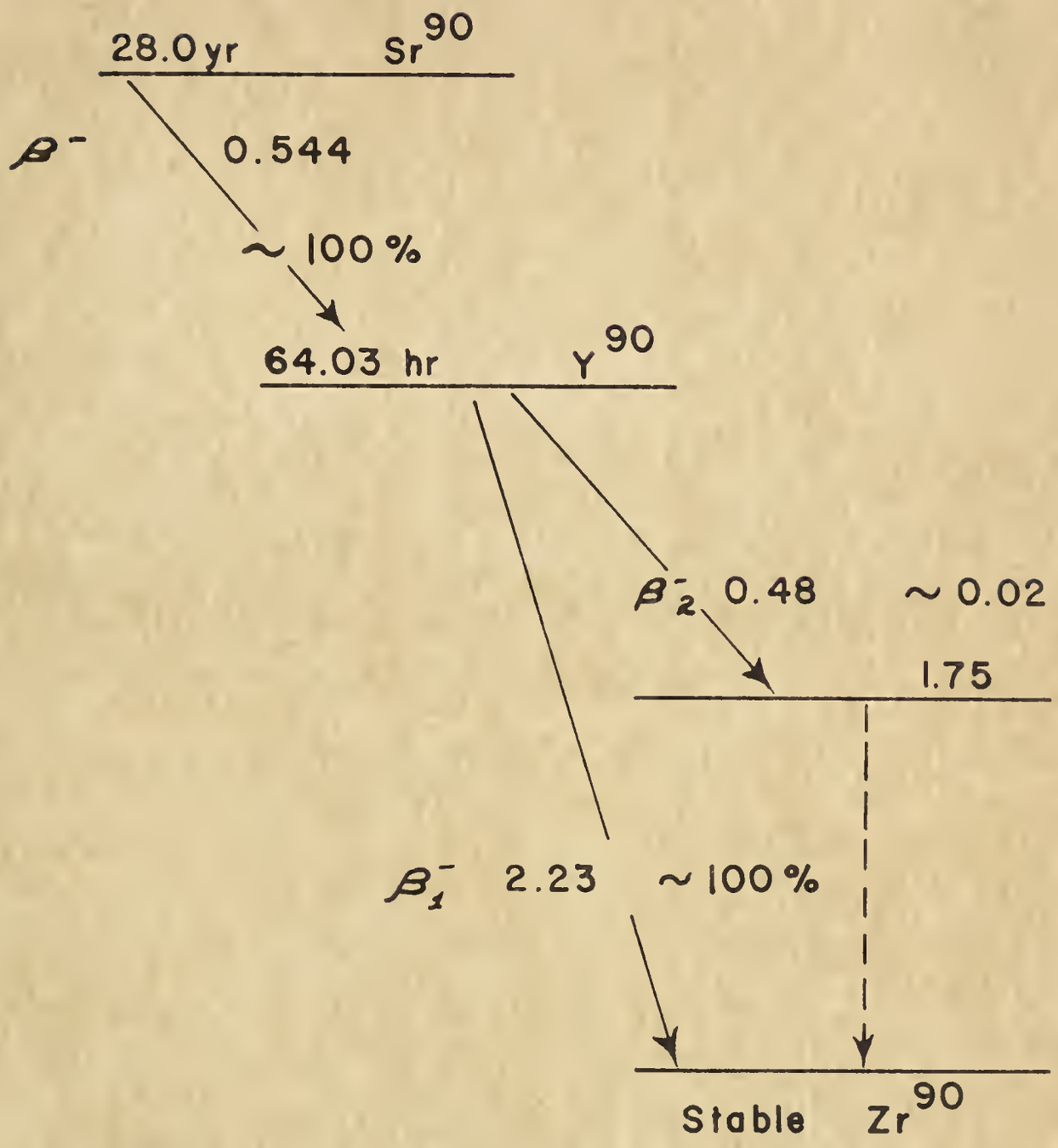
1. Ivy, A.C., Grossman, M. I., & Bachrach, H.: Peptic Ulcer; Philadelphia, 1952, The Blakiston Company.

2. Method, H.L.: History of the Development of the Surgical Treatment of Peptic Ulcer; Surgical Clinics of North America, Feb. 1954: 63.

Chapter 2 INTRODUCTION

Yttrium is a rare, grey, lustrous metal, always found in combination and associated in nature with the rare earth minerals, particularly gadolinite, xenotime, yttrotantalite, and polycrase. It was discovered in 1794 by the Finnish chemist Gadolin in the mineral gadolinite from a feldspar quarry at Ytterby near Stockholm in Sweden. In the periodic table of elements it occurs in the 3B group beneath scandium and above the lanthanides. Although yttrium is not a rare earth element, it is frequently considered as being one since it possesses chemical and physical properties similar to those of the rare earths. Yttrium has an atomic number of 39, an atomic weight of 88.92 and a valence of plus 3. It is soluble in dilute acids and forms a great many compounds closely resembling those of aluminum. Yttrium is not a normal constituent of the mammalian diet, yet a knowledge of the metabolism of yttrium is important because radioactive isotopes of yttrium are important fission products, and one of them, yttrium^{90}, is produced by the radioactive decay of strontium^{90}. The deleterious effects of the presence of strontium^{90} in living mammalian tissue is largely due to the radiation emitted by decay of yttrium^{90}, the daughter product of strontium^{90}. Yttrium^{90} has a half-life of only 64.0 hours and decays by emission of a powerful beta particle to form stable zirconium^{90}. (See Figure 1).

FIGURE I



DECAY SCHEME

At the Oak Ridge National Laboratory,[20] Oak Ridge, Tennessee, yttrium^{90} is produced as follows: it is extracted from the parent strontium^{90} in strontium chloride solution by use of tributyl phthalate from which the yttrium^{90} is re-extracted using strong hydrochloric acid. This mixture of hydrochloric acid with a large quantity of yttrium^{90} and a very small amount of strontium^{90} is passed through a Dowex 50 resin column which retains both of these ions permitting other materials to pass through. The yttrium^{90} is separated from the strontium^{90} on the column by eulting with 0.2 molar citric acid at pH of three. The eluate is taken to dryness and fumed with nitric acid at least twice to destroy any organic material present. The resultant product is then dissolved in weak hydrochloric acid. It consists of carrier free yttrium^{90} chloride with very small amounts of strontium^{90} chloride (of the order of 1×10^{-4} millicuries of strontium^{90} per millicurie of yttrium^{90}). It is then made up to catalogue specifications and is ready for shipment.

Another method for the production of yttrium^{90} is by the neutron bombardment of yttrium^{89}. Yttrium^{89} is the only naturally occurring isotope of yttrium and when it is placed in the neutron flux of an atomic pile, the following reaction occurs: Y^{89} ($n\gamma$) Y^{90}. Yttrium^{90} produced by this method always has varying amounts of carrier with it, and the degree of contamination by other radioactive substances is dependent upon the purity of the original compound. Yttrium^{90} is not available from Canadian sources.

The recent development of the chemistry of the chelating agents has provided an important means for studying the metabolism of many minerals. Chelating agents are complexing reagents which form stable

and frequently soluble complexes with metal ions. The chemical, physical and hence biological properties of a metal in aqueous solution are those of its ions. Chelation results in the suppression of these properties and the establishment of others which are characteristic of the metal complex. In aqueous solutions, the co-ordination tendency of metal ions is limited to hydration, and these water molecules are more or less loosely bound to the metal such that the negative end of the water dipole is directed towards the positive metal ion. The co-ordination number of a metal is equal to the maximum number of groups which may be simultaneously bound to it. Most metals have co-ordination numbers of 4 or 6, some of the heavy metals with higher valences have a co-ordination number of 8, and a few have co-ordination number of 2. When the co-ordinated water molecules surrounding a metallic ion in an aqueous solution are replaced by other molecules or ions, the resulting substance is called a metal complex or metal co-ordinate compound, and the group which replaces the water molecules is called the ligand. If the co-ordinated atoms of the ligands are bound to each other as well as to the metal ion, the resulting complex is a metal chelate compound. The binding between the metal and ligand may vary considerably. It may be primarily electrostatic, or it may be covalent, or intermediate between the two. Regardless of the type of bond, the function of the ligand is always the donation of electron pairs to the metal. The chelating chemical may contain two or more electron donor groups. A ligand containing two donor groups is said to be bidentate. Similarly, ter, quadri, quinti, and hexadentate ligands bind the metal in 3, 4, 5, and 6 positions respectively. Ethylenediaminetetraacetic acid (EDTA), hydroxyethylethylenediaminetriacetic acid (HEDTA), and diaminocyclohexanetetraacetic acid (CDTA) are hexadentate ligands. When the number

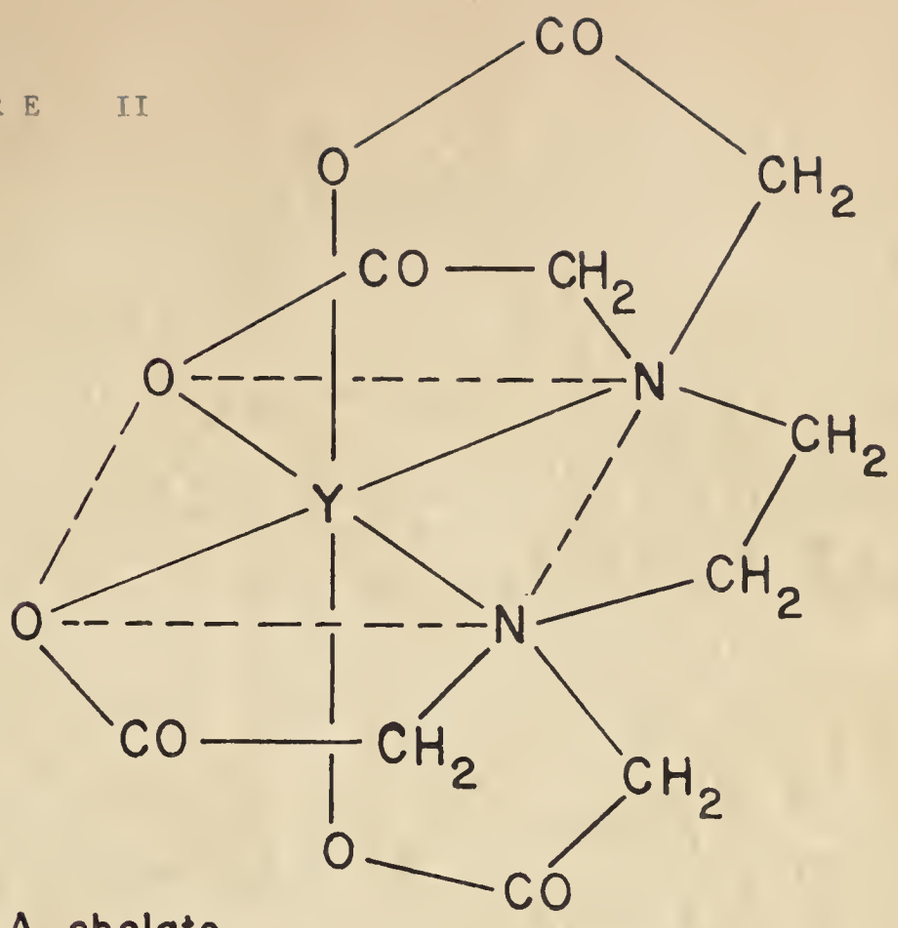
of donor groups of the ligand equal the co-ordination number of the metal, complex formation occurs in a single step, and the reaction may be considered simply the combination of two simple constituents in solution. Dihydroxyethyl glycine (DHEG) is quadridentate, and diethylenetriaminepentaacetic acid (DTPA) has eight electron donor groups. Yttrium has a co-ordinate number of 6 and combines stoichiometrically in a 1:1 ratio with each of the above chelates. Yttrium ion reacts with these chelating agents to form the following complexes: Fig. II, Fig. III, Fig. IV, Fig. V and Fig. VI.

In aqueous solutions at a pH above 6.0, yttrium is precipitated as the hydroxide. However, when combined with the aforementioned chelates the resulting metal complex is soluble. Many of the properties of the yttrium ion in aqueous solution become altered by chelation and the properties of the metal chelate compound will be dependent upon the structure of the ligand. If the metal chelate is quite stable, the reactions of the yttrium ion with various reagents may be greatly altered or completely blocked. Even when weak highly dissociated metal chelates are formed, some of the common properties of the yttrium ion such as color, solubility and oxidation reduction potential, may be altered considerably. Thus it is not surprising that the metabolism of chelated yttrium is quite different from that of the yttrium salts.

REFERENCES

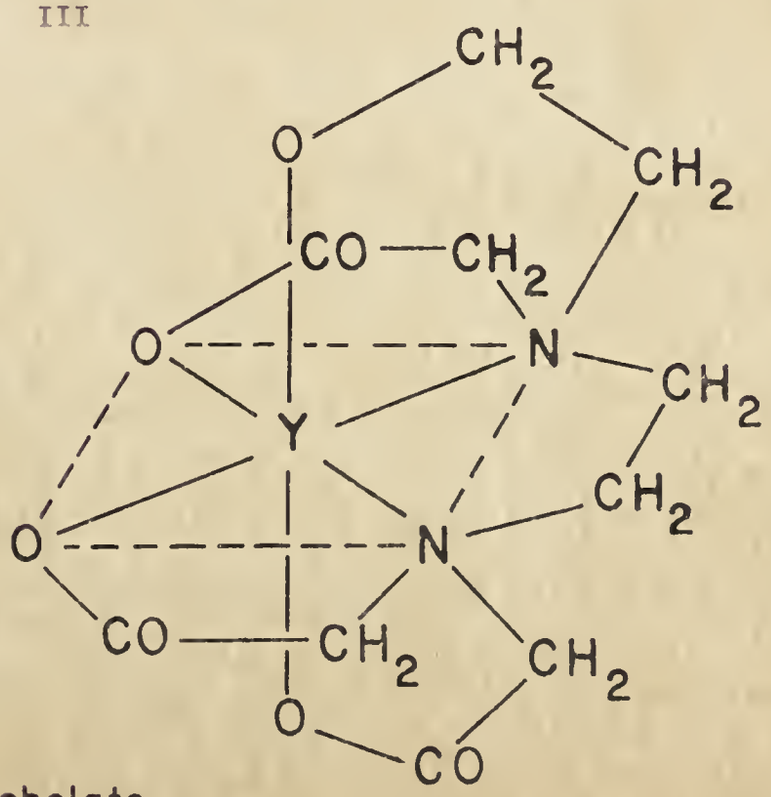
1. Encyclopaedia Britannica, Chicago, 1958, Encyclopaedia Britannica, Inc.
2. Chabarek, S., and Martell, A. E., Organic Sequestrating Agents, New York, 1959, John Wiley & Sons, Inc.
3. Smith, R. L., The Sequestration of Metals, London, 1959, Chapman & Hall Ltd.
4. Seven, M. J., and Johnson, L.A., Metal Binding in Medicine, Montreal, 1960, J. B. Lippincott Company.

FIGURE II



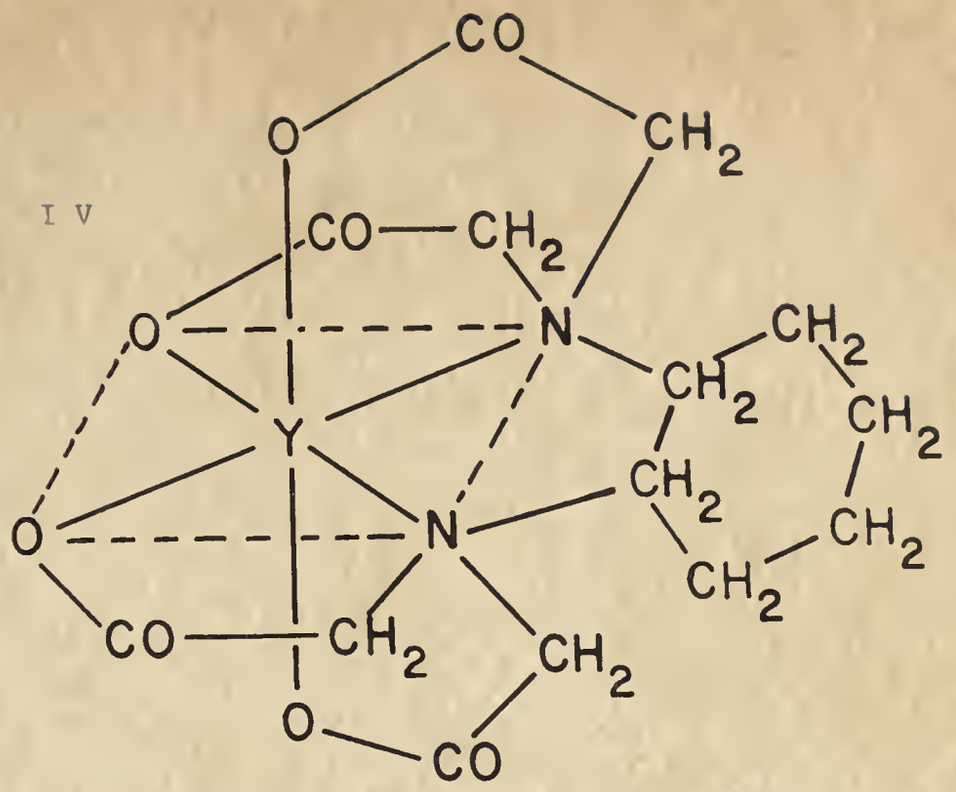
Y - EDTA chelate

FIGURE III



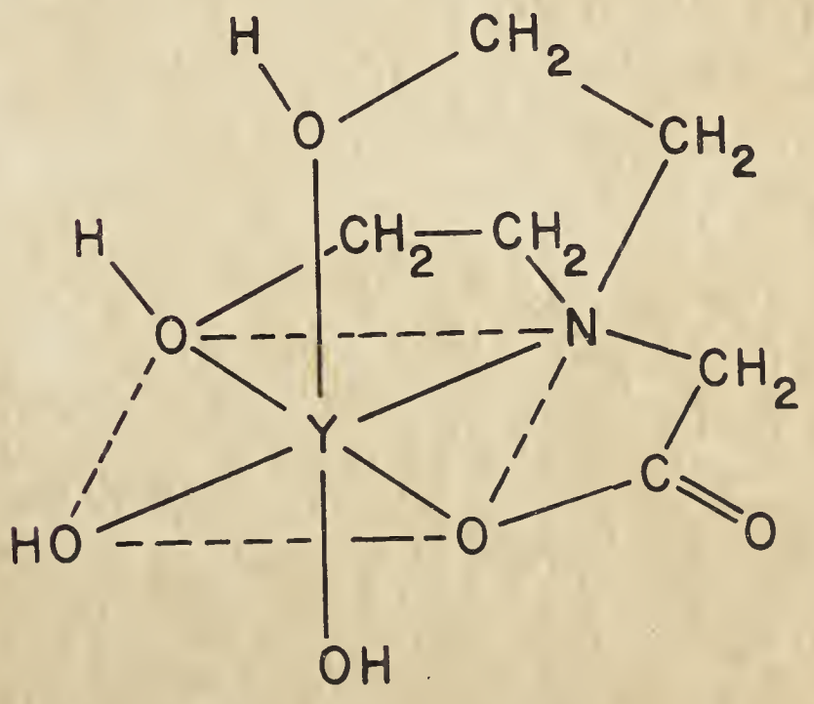
Y - HEDDA chelate

FIGURE IV



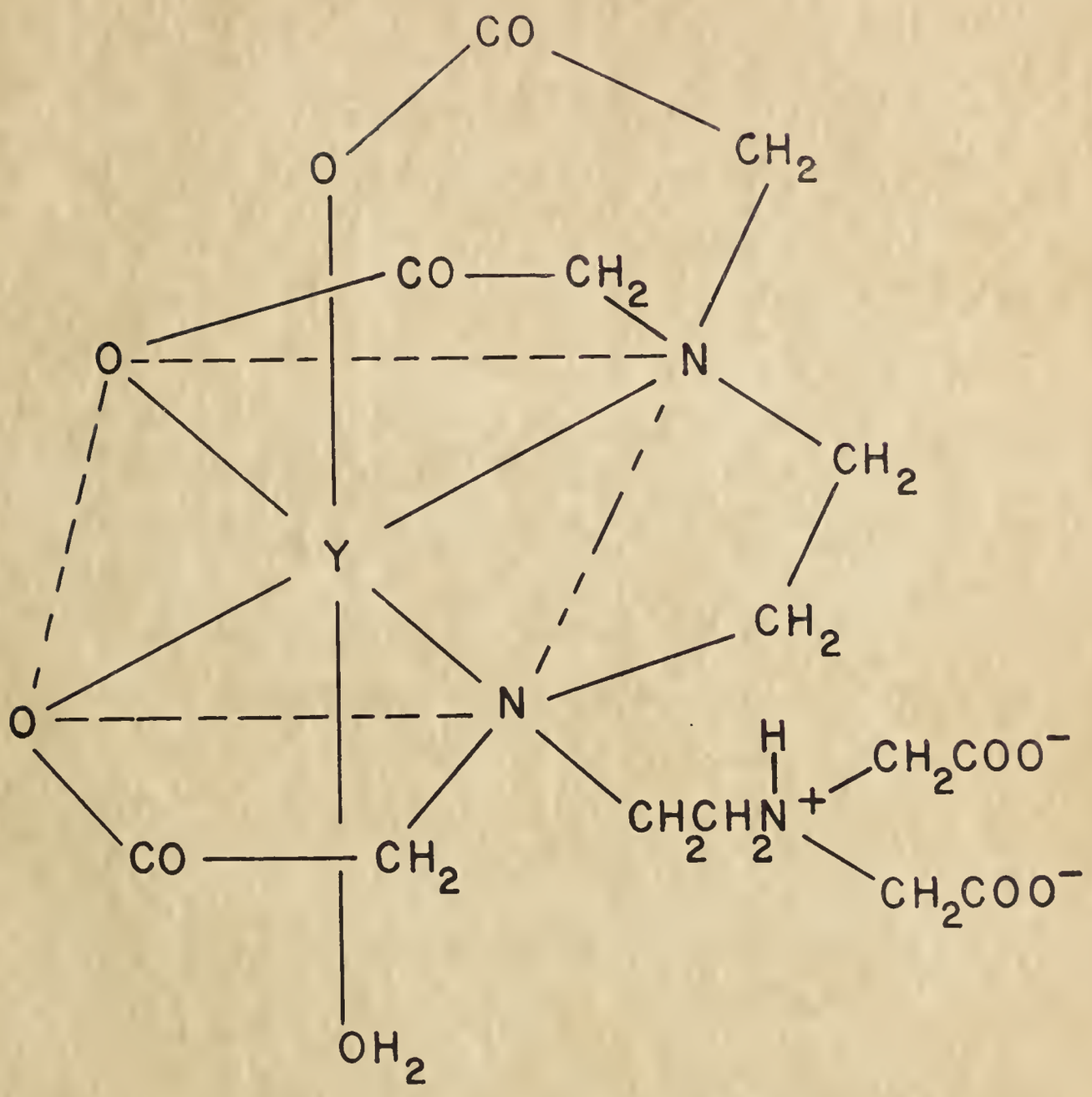
Y - CDTA chelate

FIGURE V



Y - DHEG chelate

FIGURE VI



Y - DTPA chelate

Chapter 3 REVIEW OF LITERATURE

Several groups of investigators[21,22,23,24,25,26,27] have studied the metabolism in various animals of radioactive yttrium, carrier-free and with varying amounts of non-radioactive yttrium[89] added. These studies were made with the chloride, hydroxide, citrate, nitrilotriacetic acid, and EDTA complexes of yttrium. When the total amount of yttrium was small it tended to concentrate in the skeleton. As the total amount was increased, the chloride, hydroxide and citrate salts of yttrium tended to remain in situ on intraperitoneal or intrapleural injection, or were deposited in the spleen and liver if given intravenously. Because of this tendency to remain localized and since it is a powerful beta emitter Lewin and associates[28] suggested that yttrium[90] be used as a means of reducing pleural and ascitic effusions of carcinomatosis.

In 1955 Dudley and Greenberg[19] published the first of three articles on the "Influence of Chelates of the Metabolism of Radio Yttrium". They studied the effects of different chelates, varying chelate-metal ratios and varying quantities of carrier on the metabolism of yttrium in rats, rabbits, dogs and humans. They were interested in bone metabolism and undertook to determine if yttrium could be selectively concentrated in proliferating bone.

In rats, the subcutaneous injection of yttrium chloride and yttrium complexed with citrate, EDTA, HEDTA, HEDDA (dihydroxyethylethylene-diaminediacetic acid), and DHEG showed that the yttrium chloride remained in situ, the yttrium citrate was concentrated in the liver, spleen and kidneys, and the yttrium chelate compounds showed varying

degrees of affinity for bone. The intravenous injection into rabbits of citrate and HEDDA yttrium complexes at pH 6.5 to 7.0 resulted in a high concentration of yttrium in the liver, spleen and bone marrow at 48 hours. Intravenous injections of HEDTA, EDTA, and DHEG yttrium complexes resulted in a low concentration of yttrium to the liver, spleen, and bone marrow and produced high cortical bone to marrow ratios. The amount of carrier in these studies was 0.30 milligrams of yttrium per kilogram of body weight, and the amount of chelate was twice the minimum amount required to prevent precipitation of yttrium hydroxide at pH 6.5 to 7. When the amount of EDTA, HEDTA, and HEDDA was increased to four times the minimal amount there was a marked uptake of yttrium by the ribs, cranium and trabecular bone, particularly in those animals treated with HEDTA. Trabecular bone to marrow ratios ranged from 9.6 for EDTA to 40.7 for HEDTA.

In another series of rabbits only the minimum quantity of HEDTA necessary to complex yttrium was used. The carrier level was maintained at 0.40 milligrams of yttrium per kilogram of body weight. Under these conditions only moderate amounts of yttrium were deposited in bone; the stomach, however, contained a high concentration of yttrium and stomach to marrow ratios at 48 hours exceeded 14 to 1. Of the chelating agents tested HEDTA promoted greatest bone deposition of yttrium and an excess of chelate promoted high bone to marrow ratios.

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In 1956 Dudley and Greenberg published the second article on the metabolism of radio yttrium. They studied the early tissue distribution

and the influence of dose on distribution in rabbits, and the distribution and fate of yttrium in the dog. In rabbits the intravenous administration of yttrium solution at pH 6.5 to 7.0 chelated with a minimum amount of HEDTA produced a stomach to marrow ratio at 24 hours of about 70 to 1, and at 48 hours about 20 to 1. High chelate concentration favoured deposition in bone, low chelate concentration favoured deposition in the stomach. The carrier level was 0.24 milligrams of yttrium per kilogram of body weight. Examination of various parts of the stomach showed that the yttrium was concentrated in the wall of the fundus. Rabbits were injected intravenously with yttrium chelated with five times the minimum amount of HEDTA and the carrier dose was varied from 0.08 to 5.1 milligrams of yttrium per kilogram of body weight. High carrier levels promoted deposition in liver and bone marrow, low carrier levels favoured deposition in trabecular bone with considerable quantities in the stomach, liver and cortical bone.

Studies of the distribution of yttrium in dogs following intravenous administration of yttrium^{90} chelated with five times the minimum amount of HEDTA indicated that at a carrier level of 0.05 to 0.08 milligrams of yttrium per kilogram of body weight, yttrium was deposited in all tissues but with the greatest amount in the stomach mucosa, with stomach to marrow ratios as high as 14 to 1. The relative amounts in the pyloric and fundal mucosa compared to the amounts in the fundal muscle were 7 and 12 respectively.

In 1958 Dudley and Greenberg[30] published the third article of the series. They found that the lethal dose for rabbits was from 3.5

to 4.5 millicuries of yttrium^{90} per kilogram of body weight. In dogs the lethal dose was found to be 1.5 to 2.5 millicuries of yttrium^{90} per kilogram of body weight. Yttrium^{90} solution chelated with HEDTA was administered intravenously to 3 patients. Because of the varying times of death, general conditions of the patients, and different carrier levels, the results were non-conclusive. However, in general, they showed that low carrier levels of yttrium (0.05 milligrams of yttrium per kilogram of body weight) favoured deposition in stomach and bone. High carrier levels (0.20 milligrams of yttrium per kilogram of body weight) increased the amount in liver and spleen.

Dudley showed that there was a species difference between the dog and the rabbit. Under the conditions of low carrier (0.03 to 0.08 milligrams per kilogram) and excess chelate, the rabbit showed greatest deposition in the trabecular bone, whereas the dog showed greatest concentration in the acid bearing portion of the stomach.

In an attempt to explain this species difference, Dudley postulated that "the greater selective deposition of yttrium observed in the stomach of the dog may be due to the fact that carnivores have a lower gastric pH than herbivores (rabbit) ..... As the pH is reduced the stability of the metal chelate bond decreases. For the common metals this loss of efficiency is not apparent until the pH drops below 7.5. Below this pH metal such as calcium and magnesium are not complexed efficiently. Therefore, more chelate is necessary to preserve their solubility. As the pH drops below 5.0 the strength of the metal chelate bond is not great enough to prevent precipita-

tion by the common precipitating agents. Since there is competition for yttrium between the natural complexing agents of the body, (i.e., protein amino acids, citrate, etc.), and the exogenous chelate, it is postulated that in the mucosa of the stomach (where H ion is secreted) the pH may be sufficiently low to split the chelate and cause precipitation of the yttrium in such forms as the proteinate with resultant localization".

The selective deposition of radioactive yttrium in the gastric mucosa may be of clinical significance for it may mean that malignant neoplasms of the stomach and the hypersecretion of the peptic ulcer syndrome may be treated in this fashion. Dudley made no attempt to selectively deposit radioyttrium in the gastric mucosa; in fact, he was more interested in keeping it out of the gastric mucosa and depositing it in trabecular bone, for he hoped that malignant disease of the bone could be treated by the deposition of yttrium^{90} in that tissue.

Chapter 4 AIM OF EXPERIMENT

It was in an attempt to prove Dudley's hypothesis that gastric deposition of yttrium is dependent upon gastric pH, and to investigate the effects of various chelates, chelate concentration and carrier concentration on the gastric deposition of yttrium that the following experiment was undertaken.

If the deposition of yttrium in gastric mucosa is dependent upon local pH, then the maintenance of a constant low intragastric pH should increase the deposition of yttrium in that tissue. The absorption of histamine from a subcutaneous or intramuscular injection of a mixture of histamine, beeswax and mineral oil produces a profound gastric secretory response with the maintenance of a very low intragastric pH for 36 to 48 hours after the injection. The injection of beeswax and mineral oil without the histamine has no effect on gastric secretion.

Chapter 5 EXPERIMENTAL DESIGN

The experiment was designed as a simple factorial experiment, the statistical analysis of the data to be by the analysis of variance. It was planned that carrier free yttrium^{90} and yttrium^{90} with added carrier, as well as five different chelates, each at two different levels be tested.

Dogs and guinea pigs were chosen as the experimental animals because of their availability and their known gastric secretory response to histamine.[31] The entire experiment was performed on dogs and repeated on guinea pigs. The dogs were divided into two groups; one received histamine, the other did not. These were further divided to receive either carrier free yttrium^{90} or yttrium^{90} with added carrier. The effect of yttrium^{90} alone and in combination with each of five chelates was investigated and for animals receiving yttrium^{90} with added carrier, two concentrations of each chelate were used. The overall experimental plan is shown in table One.

Reagents and Equipment:

Yttrium^{90}solution carrier free. *

Yttrium^{90}solution with added carrier. ** (0.25 milligrams per millileter)

Yttrium chloride.

Chelates.
- disodium ethylenediaminetetraacetic acid (EDTA)
- dihydroxyethyl glycine *** (DHEG)
- diaminocyclohexanetetraacetic acid *** (CDTA)
- hydroxyethylethylenediaminetriacetic acid *** (HEDTA)
- dihydroxyethylenetriaminepentaacetic acid *** (DTPA)

* Oak Ridge National Laboratory, Oak Ridge, Tennessee
** C. H. Frosst and Co., Montreal
*** Compliments of Geigy Industrial Chemicals, Ardsley, New York

Bromthymol blue, 0.1% in 50% ethyl alcohol.

2.5 Normal sodium hydroxide.

Histamine, mineral oil, beeswax mixture:

	Dogs	Guinea Pigs
Mineral Oil	16.0 milliliters	4.0 milliliters
Beeswax	4.0 grams	1.0 grams
Histamine dihydrochloride (anhydrous)	492 milligrams	41 milligrams
Concentration	15 milligrams histamine as base/milliliter	5 milligrams histamine as base/milliliter
Dose:	1 milliliter intramuscularly	0.2 milliliters intramuscularly

Heat beeswax until molten, add mineral oil and finely powdered histamine dihydrochloride and stir until cool to form a homogenous mixture.

Mineral oil, beeswax mixture:

Mineral oil	20.0 milliliters
Beeswax	5.0 grams

Heat beeswax until molten, add mineral oil and stir until cool.

Dose: Dogs 1 milliliter intramuscularly
Guinea pigs 0.2 milliliters intramuscularly

Diethyl ether.

Nembutal.

Alcoholic sodium hydroxide: 900 milliliters of 95% ethyl alcohol containing 100 grams of sodium hydroxide dissolved in 100 milliliters of water

Concentrated hydrochloric acid.

0.1 Normal hydrochloric acid.

Syringes: 10 cubic centimeters.
1 cubic centimeters.

Surgical instruments as required.

Annular 10 milliliter liquid geiger tube and lead well.

Tracerlab Scalar.

Laboratory glassware as required.

REFERENCES

1. Goulden, C. H., Methods of Statistical Analysis, New York, 1952. John Wiley & Sons, Inc.

2. Snedecor, G. W., Statistical Methods Applied to Experiments in Agriculture and Biology. Ames, Iowa, 1956. Iowa State College Press.

Chapter 6 EXPERIMENTAL PROCEDURE

Healthy adult mongrel dogs weighing from 6 to 12 kilograms were divided into six groups, each composed of six dogs. The first four groups (24 dogs) received yttrium^{90} with carrier, the last two groups (12 dogs) received carrier free yttrium^{90}. A plan of the experiment is presented in Table I.

One milliliter of the histamine-mineral oil-beeswax mixture was administered by a deep intramuscular (gluteal) injection 1 to 2 hours prior to the injection of the yttrium^{90} solution. Those dogs which did not get histamine received one milliliter of the mineral oil-beeswax mixture by deep intramuscular injection.

Yttrium^{90} With Added Carrier:

The first four groups of dogs received yttrium^{90} solution with added carrier, purchased from C. H. Frosst & Co., Montreal. Although the specific activity varied from 67 to 267 microcuries per milligram of yttrium, the concentration of yttrium remained constant at 0.25 milligrams per milliliter. Each animal received a total of 2.1 milligrams of yttrium and, depending upon the delay between assay time and the time of injection, from 150 to 250 microcuries of yttrium^{90}. From each shipment of yttrium^{90} one milliliter of solution was removed, a few milliliters of 1 N yttrium^{89} chloride added and then diluted to 1:5000 with dilute hydrochloric acid and set aside as the standard. The presence of carrier and the maintenance of an acid pH decreased the absorption of yttrium^{90} onto the glass walls of the container to almost zero.

EXPERIMENTAL PLAN

TABLE I — YTTRIUM^{90} WITH ADDED CARRIER

				Chelate Concentration
Group 1	Dog No. 1	no histamine	no chelate	
	2	no histamine	no chelate	
	3	histamine	no chelate	
	4	histamine	no chelate	
	5	no histamine	EDTA	low concentration
	6	histamine	EDTA	low concentration
Group 2	Dog No. 7	no histamine	EDTA	high concentration
	8	histamine	EDTA	high concentration
	9	no histamine	CDTA	low concentration
	10	histamine	CDTA	low concentration
	11	no histamine	CDTA	high concentration
	12	histamine	CDTA	high concentration
Group 3	Dog No. 13	no histamine	DTPA	low concentration
	14	histamine	DTPA	low concentration
	15	no histamine	DTPA	high concentration
	16	histamine	DTPA	high concentration
	17	no histamine	HEDTA	low concentration
	18	histamine	HEDTA	low concentration
Group 4	Dog. No.19	no histamine	HEDTA	high concentration
	20	histamine	HEDTA	high concentration
	21	no histamine	DHEG	low concentration
	22	histamine	DHEG	low concentration
	23	no histamine	DHEG	high concentration
	24	histamine	DHEG	high concentration

YTTRIUM^{90} CARRIER FREE

Group 5	Dog No. 25	no histamine	no chelate
	26	histamine	no chelate
	27	no histamine	EDTA
	28	histamine	EDTA
	29	no histamine	CDTA
	30	histamine	CDTA
Group 6	Dog No. 31	no histamine	DTPA
	32	histamine	DTPA
	33	no histamine	HEDTA
	34	histamine	HEDTA
	35	no histamine	DHEG
	36	histamine	DHEG

A low concentration of chelate was an amount equimolecular to the amount of yttrium ion present in the solution; a high concentration of chelate was five times this amount. To prevent precipitation of yttrium hydroxide, the yttrium^{90} solution was maintained at a pH of about three until after the addition of the proper amount and variety of chelate and just prior to injection into the experimental animals, at which time the solution was made alkaline to bromthymol blue with 2.5 Normal sodium hydroxide. The solution was then injected intravenously via the anterior cubital vein with a 10 cubic centimeter syringe and a No. 21 needle. The dogs were kept in wire floored cages, and allowed water adlibitum but no food. Twenty-four hours after injection of the yttrium^{90} solution, the animals were sacrificed by intravenous injection of Nembutal and aliquots of various tissues removed for analysis.

Carrier Free Yttrium^{90}

The preparation of carrier free yttrium^{90} chelate solution for injection, preparation of the standard and handling of the experimental animals was the same as for the previous four groups. Carrier free yttrium^{90} as yttrium^{90} chloride in dilute hydrochloric acid, was purchased from Oak Ridge National Laboratory, Oak Ridge, Tennessee; and according to the assay sheet which accompanied each shipment, the total solids in solution varied from one to five milligrams per millicurie of yttrium^{90}. Oak Ridge National Laboratory was unable to supply an accurate chemical analysis of the solution, therefore, to determine the amount of chelate necessary to complex all of the metal ions in the solution, it was assumed that yttrium ion constituted one-half of the

total solids present. Each animal received 135 microcuries of yttrium^{90}.

The following tissues were analyzed for yttrium^{90} content; spleen, liver, bone marrow (rib), dry bone (rib), gastric fundus muscle, gastric fundus mucosa, gastric antrum muscle, gastric antrum mucosa.

Preparation of Sample

The tissue aliquots were placed in tared 50 milliliter Erlenmeyer flasks, weighed, and approximately one milliliter of 1 Normal yttrium^{89} chloride solution added. Ten milliliters of alcoholic sodium hydroxide solution were added to each flask, and they were placed on a hot plate and maintained at about 60^{o} C. overnight. By the following morning all the tissues except the bones had dissolved. The bones were removed from each flask, rinsed thoroughly with distilled water and dried, after which they were weighed and dissolved in 10 milliliters of 0.1 Normal hydrochloric acid. The difference in weight between the original bone and the dry bone was assumed to be the weight of the marrow. Two milliliters of concentrated hydrochloric acid were added to the tissue alcoholic sodium hydroxide solutions. This was sufficient to bring the pH of the solution to about one, thus ensuring that all yttrium would be in solution. The activity of the samples were measured using a 10-milliliter liquid geiger tube, lead well and a Tracerlab scalar. The operating voltage of the geiger tube was set at 75 volts above the threshold voltage and was not changed throughout the entire experiment. The same liquid geiger tube was used for each sample. Rinsing the liquid geiger tube once with 0.1 Normal hydrochloric acid and five times with water after each sample reduced the water blank to background

level. The samples were counted for sufficient time such that the percent standard error was limited to a maximum of 4%. This usually required from one to ten minutes. The background was counted using a water blank for several hours prior to counting the samples.

The experimental procedure as described previously, was repeated using guinea pigs as the experimental animal. For those guinea pigs receiving histamine, the dose was five milligrams of histamine, calculated as base, in 0.2 milliliters of histamine, mineral oil, beeswax mixture, administered intramuscularly in the right hind leg. The other guinea pigs received a dose of 0.2 milliliters intramuscularly (right hind leg) of the mineral oil- beeswax mixture. The dose of yttrium per guinea pig was 0.15 milligrams and the dose of yttrium^{90} varied from 10 to 18 microcuries. The following tissues were analyzed for yttrium content: stomach (whole), spleen (whole), kidney (whole), liver, bone and bone marrow (midshaft of left tibia).

A coincidence loss curve was plotted by counting serial dilutions of five microcuries of yttrium^{90} and this graph was applied to correct all the tissue and standard counts for coincidence loss.

Analysis of Results

The data as counts per minute per gram of tissue were transformed to the common logarithm and the statistical analysis performed on the transformed data.

Chapter 7 RESULTS AND DISCUSSION

Tables II, III, IV and V are the data as obtained in all the experiments. The counts as shown in these tables times one thousand give the counts per minute per gram of tissue obtained after correction for decay, coincidence loss and assay variations. The results of using yttrium^{90} with added carrier are shown for dogs in Table II and for guinea pigs in Table IV. The results of using carrier free yttrium^{90} are shown for dogs in Table III and for guinea pigs in Table V. The results of histamine and no histamine treated animals on the deposition of yttrium in various tissues is shown in Tables VI and VII. t tests to determine the significance of difference between histamine and no histamine treated animals are included in these tables. From these it is apparent that there is no significant difference between the yttrium concentration in the tissues of animals which received histamine and those which did not. Gastric deposition of yttrium is not dependent upon the lowered intragastric pH produced by histamine. The results of high and low chelate concentration on the deposition of yttrium in the various tissues is shown in Tables VIII and IX. Included in these tables are t tests to determine the significance of differences between high and low chelate concentrations. The differences are not significant in any of the tissues examined.

Although the experiment was originally planned as a factorial with three replicates, it was later changed to the form as shown in Table I. Upon completion of the t tests as reported in Tables VI, VII, VIII and IX it was accepted that neither histamine nor chelate concentration had

EXPERIMENTAL RESULTS OF DOGS RECEIVING YTTRIUM^{90} WITH ADDED CARRIER

TABLE II

counts per minute per gram of tissue x 1000

Dog	Carrier	Histamine	Chelate	Concentration of Chelate	Fundus Mucosa	Fundus Muscle	Antrum Mucosa	Antrum Muscle	Spleen	Liver	Kidney	Bone Marrow	Dry Bone
No. 1	yes	no	none		0.36	0.25	1.52	0.42	131.59	39.13	4.95	2.44	17.34
2	yes	no	none		0.20	0.17	0.23	0.30	138.17	50.59	0.99	0.00	4.30
3	yes	yes	none		0.28	0.62	1.42	0.15	92.38	63.94	5.68	2.94	24.37
4	yes	yes	none		0.54	0.00	0.30	0.21	16.31	19.74	0.36	0.13	1.99
5	yes	no	EDTA	low	37.66	0.38	17.43	0.90	1.72	2.26	3.30	1.28	5.90
6	yes	yes	EDTA	low	40.22	1.26	32.26	1.42	2.84	5.75	5.73	2.85	16.10
7	yes	no	EDTA	high	18.21	0.17	4.11	0.73	0.55	0.46	4.56	3.21	11.58
8	yes	yes	EDTA	high	28.18	0.16	8.47	1.29	0.44	1.18	4.49	0.87	4.14
9	yes	no	CDTA	low	0.06	0.07	0.10	0.05	1.22	1.80	3.83	0.20	3.34
10	yes	yes	CDTA	low	0.10	0.08	0.14	0.06	3.15	2.60	3.93	0.28	2.67
11	yes	no	CDTA	high	0.08	0.07	0.10	0.06	2.77	1.59	3.37	1.17	3.78
12	yes	yes	CDTA	high	0.18	0.09	0.39	0.16	2.84	0.56	2.78	0.17	3.40
13	yes	no	DTPA	low	0.09	0.05	0.11	0.05	0.77	0.61	4.00	0.13	0.21
14	yes	yes	DTPA	low	0.06	0.02	0.08	0.02	0.54	0.89	2.47	0.00	0.26
15	yes	no	DTPA	high	0.14	0.27	0.08	0.06	0.16	0.27	1.70	0.00	0.06
16	yes	yes	DTPA	high	0.13	0.04	0.07	0.00	0.23	0.42	1.55	0.00	0.20
17	yes	no	HEDTA	low	6.56	1.46	10.84	2.80	68.75	26.72	7.93	0.34	23.90
18	yes	yes	HEDTA	low	4.92	1.11	42.15	5.07	41.79	47.69	12.73	0.69	20.41
19	yes	no	HEDTA	high	7.96	1.98	50.23	3.05	17.05	11.77	5.29	0.98	18.39
20	yes	yes	HEDTA	high	10.07	2.06	44.12	2.07	8.57	10.66	6.66	1.74	27.46
21	yes	no	DHEG	low	0.17	0.26	0.32	0.38	206.53	46.98	2.17	0.20	4.05
22	yes	yes	DHEG	low	0.08	0.03	0.09	0.08	45.64	68.77	1.64	0.33	3.57
23	yes	no	DHEG	high	1.17	0.60	1.68	0.45	81.76	37.23	5.79	0.80	20.24
24	yes	yes	DHEG	high	1.00	0.75	1.14	0.54	92.80	49.57	19.15	2.54	32.49

EXPERIMENTAL RESULTS OF DOGS RECEIVING CARRIER FREE YTTRIUM^{90}

TABLE III

counts per minute per gram of tissue x 1000

Dog	Carrier	Histamine	Chelate	Concentration of Chelate	Fundus Mucosa	Fundus Muscle	Antrum Mucosa	Antrum Muscle	Spleen	Liver	Kidney	Bone Marrow	Dry Bone
No. 25	no	no	none		3.02	0.71	2.10	1.33	1.90	15.00	6.65	1.91	13.38
26	no	yes	none		1.05	0.63	3.46	1.10	0.32	7.09	5.91	1.21	22.48
27	no	no	EDTA		1.48	0.87	6.40	0.77	3.58	5.37	15.33	0.74	22.02
28	no	yes	EDTA		1.01	0.64	2.27	1.58	0.43	7.11	10.87	1.29	32.51
29	no	no	CDTA		0.09	0.12	0.12	0.15	0.18	0.41	2.37	0.33	4.76
30	no	yes	CDTA		0.45	0.24	0.60	0.26	0.27	0.58	9.12	0.70	6.77
31	no	no	DTPA		0.02	0.01	0.02	0.01	0.01	0.03	0.75	0.01	0.03
32	no	yes	DTPA		0.02	0.01	0.02	0.01	0.01	0.04	0.54	0.01	0.03
33	no	no	HEDTA		0.84	0.80	1.75	4.74	7.36	12.00	6.17	1.74	24.86
34	no	yes	HEDTA		0.97	0.44	0.76	0.78	3.69	11.15	3.03	1.51	24.35
35	no	no	DHEG		0.42	0.36	0.52	0.37	4.31	8.76	1.31	0.17	6.84
36	no	yes	DHEG		0.60	0.14	0.47	0.23	1.34	6.91	1.08	0.26	5.86

EXPERIMENTAL RESULTS OF GUINEA PIGS RECEIVING YTTRIUM^{90} WITH ADDED CARRIER

TABLE IV

counts per minute per gram of tissue x 1000

Guinea Pig	Carrier	Histamine	Chelate	Concentration of Chelate	Stomach	Spleen	Kidney	Liver	Bone Marrow	Dry Bone
No. 1	yes	no	none		1.46	208.61	12.81	137.73	4.04	32.34
2	yes	no	none		1.58	269.75	11.50	131.60	2.65	29.11
3	yes	yes	none		1.20	67.78	5.42	113.09	3.87	25.58
4	yes	yes	none		1.82	97.01	11.72	122.80	1.95	20.38
5	yes	no	EDTA	low	1.37	4.10	6.26	3.81	4.64	19.25
6	yes	yes	EDTA	low	1.40	1.97	7.54	1.73	3.56	22.81
7	yes	no	EDTA	high	0.23	2.55	4.60	1.43	1.45	10.18
8	yes	yes	EDTA	high	0.51	1.23	3.67	1.48	5.24	35.44
9	yes	no	CDTA	low	0.32	4.78	3.88	4.33	0.53	2.98
10	yes	yes	CDTA	low	0.21	5.84	3.02	2.11	0.56	4.20
11	yes	no	CDTA	high	0.21	1.73	3.19	2.38	1.36	4.75
12	yes	yes	CDTA	high	0.40	1.44	2.88	1.32	0.45	3.08
13	yes	no	DTPA	low	0.14	2.35	3.83	1.84	0.00	0.17
14	yes	yes	DTPA	low	0.13	9.75	5.34	5.52	0.00	0.19
15	yes	no	DTPA	high	0.03	0.21	1.75	0.13	0.00	0.21
16	yes	yes	DTPA	high	0.03	0.40	1.35	0.11	0.00	0.22
17	yes	no	HEDTA	low	14.36	42.22	16.20	45.24	1.40	7.91
18	yes	yes	HEDTA	low	13.64	27.22	6.18	20.60	1.04	6.19
19	yes	no	HEDTA	high	20.40	51.52	12.33	35.56	2.45	12.72
20	yes	yes	HEDTA	high	18.48	11.89	11.84	15.71	2.08	15.16
21	yes	no	DHEG	low	0.82	187.41	4.40	132.85	1.51	5.97
22	yes	yes	DHEG	low	0.31	67.08	1.42	81.93	1.70	1.77
23	yes	no	DHEG	high	2.09	239.77	5.10	97.54	4.57	12.67
24	yes	yes	DHEG	high	1.79	129.43	3.69	74.26	2.53	4.88

EXPERIMENTAL RESULTS OF GUINEA PIGS RECEIVING CARRIER FREE YTTRIUM^{90}

TABLE V

counts per minute per gram of tissue x 1000

Guinea Pig	Carrier	Histamine	Chelate	Concentration of Chelate	Stomach	Spleen	Kidney	Liver	Bone Marrow	Dry Bone
No. 25	no	no	none		1.82	51.65	9.60	23.76	1.25	15.78
26	no	yes	none		1.20	39.42	14.33	33.47	1.74	19.46
27	no	no	EDTA		3.32	7.25	30.40	20.11	28.44	25.04
28	no	yes	EDTA		2.47	7.98	38.46	19.91	24.94	28.80
29	no	no	CDTA		0.23	1.24	5.94	2.08	2.24	8.18
30	no	yes	CDTA		0.30	1.38	3.48	1.68	2.21	7.58
31	no	no	DTPA		0.03	0.09	0.95	0.06	0.00	0.07
32	no	yes	DTPA		0.04	0.16	0.99	0.10	0.00	0.09
33	no	no	HEDTA		2.53	15.69	21.24	58.23	15.69	54.40
34	no	yes	HEDTA		3.21	86.91	45.80	39.56	2.80	13.23
35	no	no	DHEG		0.50	15.09	6.30	16.20	1.42	5.68
36	no	yes	DHEG		0.65	8.20	7.82	23.00	0.00	1.74

TABLE VI

t TEST TO DETERMINE SIGNIFICANCE OF DIFFERENCE BETWEEN HISTAMINE AND NO HISTAMINE TREATED DOGS

Tissue	Histamine treated dogs c./min./g. x 1000*	No histamine treated dogs c./min./g. x 1000	Calculated value of t
fundus mucosa	5.04	4.36	0.09
fundus muscle	0.46	0.48	1.06
antrum mucosa	7.68	5.43	0.21
antrum muscle	0.84	1.35	0.52
spleen	17.42	37.13	0.57
liver	16.92	14.50	0.19
kidney	5.43	4.47	0.03
bone marrow	0.97	0.87	0.14
dry bone	12.72	10.28	0.20

t at p = 0.05 for 34 D.F. is 2.03

* c./min./g. = counts per minute per gram of tissue

TABLE VII

t TEST TO DETERMINE SIGNIFICANCE OF DIFFERENCE BETWEEN HISTAMINE AND NO HISTAMINE TREATED GUINEA PIGS

Tissue	Histamine treated guinea pigs c./min./g. x 1000*	No histamine treated guinea pigs c./min./g. x 1000	Calculated value of t
stomach	2.66	2.86	0.07
spleen	31.39	61.44	0.28
kidney	9.72	8.90	0.14
liver	31.02	39.72	0.13
bone marrow	6.99	4.09	0.52
dry bone	11.71	13.74	0.30

t at p = 0.05 for 34 D.F. is 2.03

*c./min/g. = counts per minute per gram of tissue

TABLE VIII

t TEST TO DETERMINE SIGNIFICANCE OF DIFFERENCE BETWEEN LOW AND HIGH CHELATE CONCENTRATION IN DOGS

Tissue	Low chelate concentration c./min./g. x 1000*	High chelate concentration c./min./g. x 1000	Calculated value of t
fundus mucosa	8.99	6.71	0.57
fundus muscle	0.47	0.62	0.44
antrum mucosa	10.35	11.04	0.30
antrum muscle	1.08	0.84	0.07
spleen	37.30	20.72	0.08
liver	20.41	11.37	1.09
kidney	4.77	5.53	0.23
bone marrow	0.63	1.15	0.12
dry bone	8.04	12.17	0.23

t at p = 0.05 for 18 D.F. is 2.10

* c./min./g. = counts per minute per gram of tissue

TABLE IX

t TEST TO DETERMINE SIGNIFICANCE OF DIFFERENCE BETWEEN LOW AND HIGH CHELATE CONCENTRATION IN GUINEA PIGS

Tissue	Low chelate concentration c./min./g. x 1000*	High chelate concentration c./min./g. x 1000	Calculated value of t
stomach	3.27	4.42	0.23
spleen	35.27	44.02	0.91
kidney	5.81	5.04	0.21
liver	30.00	22.99	1.05
bone marrow	1.49	2.01	0.19
dry bone	7.14	9.93	0.45

t at p = 0.05 for 18 D.F. is 2.10

*c./min./g. = counts per minute per gram of tissue

an effect upon yttrium deposition in the tissues examined. Therefore, to facilitate a more complete statistical analysis of the effect of carrier and chelates, the factors histamine and chelate concentration were considered as replications. Because of the design involved these so- called replicates are incorporated into the error term in the analysis of variance. Tables X to XXIV show the results of the analysis of variance. In all tissues examined the effect of carrier was significant at either the 5% or 1% level, and the effect of chelate was significant at the 1% level.

In dogs, EDTA and HEDTA in the presence of added carrier produced a much higher gastric mucosal concentration of yttrium than CDTA, DHEG, DTPA or non-chelated yttrium. This is substantiated by the F values of 32.6 and 68.9 for fundal mucosa and antral mucosa respectively, as seen in Tables X and XII. Most of the yttrium in the stomach was in the fundal and antral mucosa; very little was in the muscle layer. In guinea pigs only HEDTA produced a significant gastric deposition of yttrium. This is substantiated by an F value of 416: (Table XIX). When carrier free yttrium^{90} was used, none of the chelates tested produced an appreciable amount of yttrium in the stomach: (Tables X, XI, XII, XIII and XIX).

TABLE X

DOG - FUNDUS MUCOSA

	D.F. *	S.S. **	M.S. ***	F	F at p=0.05	F at p=0.01
Carrier	1	0.64	0.64	8.20	4.75	9.33
Chelate	5	12.72	2.54	32.6	3.11	5.06
Carrier + chelate	5	2.35	0.67	8.59	3.11	5.06
Error + replicates	12	0.94	0.078			
TOTAL	23	17.65				

* D.F. - Degrees of Freedom ** S.S. - Sums of Squares *** M.S. - Mean Square

The effect of carrier is significant at the 5% level.
The effect of chelate is significant at the 1% level.
The interaction effect of carrier and chelate is significant at the 1% level.

TABLE XI

DOG - FUNDUS MUSCLE

	D.F.	S.S.	M.S.	F	F at p = 0.05	F at p = 0.01
Carrier	1	0.0811	0.0811	2.52	4.75	9.33
Chelate	5	6.1659	1.2332	38.3	3.11	5.06
Carrier + chelate	5	1.4258	0.2852	8.86	3.11	5.06
Error + replicates	12	0.3868	0.0322			
TOTAL	23	8.0596				

The effect of carrier is not significant at the 5% level.
The effect of chelate is significant at the 1% level.
The interaction effect of carrier and chelate is significant at the 1% level.

TABLE XII

DOG - ANTRUM MUCOSA

	D.F.	S.S.	M.S.	F	F at p = 0.05	F at p = 0.01
Carrier	1	1.1717	1.1717	25.3	4.75	9.33
Chelate	5	15.9502	3.1900	68.9	3.11	5.06
Carrier + chelate	5	2.2046	0.4409	9.52	3.11	5.06
Error + replicates	12	0.5567	0.0463			
TOTAL	23	19.8832				

The effect of carrier is significant at the 1% level.
The effect of chelate is significant at the 1% level.
The interaction effect of carrier and chelate is significant at the 1% level.

TABLE XIII

DOG - ANTRUM MUSCLE

	D.F.	S.S.	M.S.	F	F at p = 0.05	F at p = 0.01
Carrier	1.	.0880	0.0880	1.20	4.75	9.33
Chelate	5	12.2738	2.4548	33.5	3.11	5.06
Carrier + chelate	5	1.7536	0.3507	4.79	3.11	5.06
Error + replicates	12	0.8780	0.0732			
TOTAL	23	14.9934				

The effect of carrier is not significant at the 5% level.
The effect of chelate is significant at the 1% level.
The interaction effect of carrier and chelate is significant at the 5% level.

TABLE XIV

DOG - SPLEEN

	D.F.	S.S.	M.S.	F	F at p = 0.05	F at p = 0.01
Carrier	1	9.1286	9.1286	111	4.75	9.33
Chelate	5	18.2046	3.6409	44.4	3.11	5.06
Carrier + chelate	5	2.8112	0.5622	6.86	3.11	5.06
Error + replicates	12	0.9813	0.0819			
Total	23	31.1257				

The effect of carrier is significant at the 1% level.
The effect of chelate is significant at the 1% level.
The interaction effect of carrier and chelate is significant at the 1% level.

TABLE XV

DOG - LIVER

	D.F.	S.S.	M.S.	F	F at p = 0.05	F at p = 0.01
Carrier	1	1.5418	1.5418	144	4.75	9.33
Chelate	5	15.7693	3.1539	295	3.11	5.06
Carrier + chelate	5	1.4828	0.2966	27.7	3.11	5.06
Error + replicates	12	0.1285	0.0107			
TOTAL	23	18.9224				

The effect of carrier is significant at the 1% level.
The effect of chelate is significant at the 1% level.
The interaction of carrier and chelate is significant at the 1% level.

TABLE XVI

DOG - KIDNEY

	D.F.	S.S.	M.S.	F	F at p = 0.05	F at p = 0.01
Carrier	1	0.0763	0.0763	2.69	4.75	9.33
Chelates	5	1.7442	0.3488	12.3	3.11	5.06
Carrier + chelates	5	0.9995	0.1999	7.04	3.11	5.06
Error + replicates	12	0.3411	0.0284			
TOTAL	23	3.1611				

The effect of carrier is not significant at the 5% level.
The effect of chelate is significant at the 1% level.
The interaction effect of carrier and chelate is significant at the 1% level.

TABLE XVII

DOG - BONE MARROW

	D.F.	S.S.	M.S.	F	F at p = 0.05	F at p = 0.01
Carrier	1	.0185	0.0185	.112	244	6106
Chelate	5	13.3069	2.6614	16.1	3.11	5.06
Carrier + Chelate	5	0.5568	0.1114	0.674	4.68	9.89
Error + replicates	12	1.9847	0.1654			
TOTAL	23	15.8669				

The effect of carrier is not significant at the 5% level.
The effect of chelate is significant at the 1% level.
The interaction effect of carrier and chelate is not significant at the 5% level.

TABLE XVIII

DOG - DRY BONE

	D.F.	S.S.	M.S.	F	F at p = 0.05	F at p = 0.01
Carrier	1	0.0121	0.0121	1.70	4.75	9.33
Chelate	5	17.3272	3.4654	488	3.11	5.06
Carrier & Chelate	5	0.9653	0.1931	27.2	3.11	5.06
Error + replicates	12	0.0857	0.0071			
TOTAL	23	18.3903				

The effect of carrier is not significant at the 5% level.
The effect of chelate is significant at the 1% level.
The interaction effect of carrier and chelate is significant at the 1% level.

TABLE XIX

GUINEA PIG - STOMACH

	D.F.	S.S.	M.S.	F	F at p = 0.05	F at p = 0.01
Carrier	1	0.1633	0.1633	32.7	4.75	9.33
Chelate	5	10.3949	2.0790	416	3.11	5.06
Carrier + chelate	5	0.9312	0.1862	37.3	3.11	5.06
Error + replicates	12	0.0599	.0050			
TOTAL	23	11.5493				

The effect of carrier is significant at the 1% level.
The effect of chelate is significant at the 1% level.
The interaction effect of carrier and chelate is significant at the 1% level.

TABLE XX

GUINEA PIG - SPLEEN

	D.F.	S.S.	M.S.	F	F at p = 0.05	F at p = 0.01
Carrier	1	1.3933	1.3933	34.8	4.75	9.33
Chelate	5	14.7724	2.9545	73.7	3.11	5.06
Carrier + chelate	5	2.5872	0.5174	12.9	3.11	5.06
Error + replicates	12	0.4815	0.0401			
TOTAL	23	19.2344				

The effect of carrier is significant at the 1% level.
The effect of chelate is significant at the 1% level.
The interaction effect of carrier and chelate is significant at the 1% level.

TABLE XXI

GUINEA PIG - KIDNEY

	D.F.	S.S.	M.S.	F	F at p = 0.05	F at p = 0.01
Carrier	1	0.2456	0.2456	20.8	4.75	9.33
Chelate	5	3.0834	0.6167	52.3	3.11	5.06
Carrier + chelate	5	.9157	0.1831	15.5	3.11	5.06
Error + replicates	12	0.1418	0.0118			
TOTAL	23	4.3865				

The effect of carrier is significant at the 1% level.
The effect of chelate is significant at the 1% level.
The interaction effect of carrier and chelate is significant at the 1% level.

TABLE XXII

GUINEA PIG - LIVER

	D.F.	S.S.	M.S.	F	F at p = 0.05	F at p = 0.01
Carrier	1	0.2046	0.2046	3.54	4.75	9.33
Chelate	5	14.0248	2.8050	48.5	3.11	5.06
Chelate + carrier	5	3.0654	0.6131	10.6	3.11	5.06
Error + replicates	12	0.6944	0.0578			
TOTAL	23	17.9892				

The effect of carrier is not significant at the 5% level.
The effect of chelate is significant at the 1% level.
The interaction effect of carrier and chelate is significant at the 1% level.

TABLE XXIII

GUINEA PIG - BONE MARROW

	D.F.	S.S.	M.S.	F	F at p = 0.05	F at p = 0.01
Carrier	1	0.0097	0.0097	0.022	244	6106
Chelate	5	41.1417	8.2284	18.6	3.11	5.06
Carrier + chelate	5	4.7129	0.9426	2.13	3.11	5.06
Error + replicates	12	5.3130	0.4427			
TOTAL	23	51.1773				

The effect of carrier is not significant at the 5% level.
The effect of chelate is significant at the 1% level.
The interaction effect of carrier and ehelate is not significant at the 5% level.

TABLE XXIV

GUINEA PIG - DRY BONE

	D.F.	S.S.	M.S.	F	F at p = 0.05	F at p = 0.01
Carrier	1	.0356	0.0356	1.24	4.75	9.33
Chelate	5	14.8950	2.9790	104	3.11	5.06
Carrier + chelate	5	.4798	0.0960	3.34	3.11	5.06
Error + replicates	12	0.3446	0.0287			
TOTAL	23	15.7550				

The effect of carrier is not significant at the 5% level.
The effect of chelate is significant at the 1% level.
The interaction effect of carrier and chelate is significant at the 5% level.

Chapter 8 SUMMARY

1. Histamine had no effect on the deposition of yttrium in any of the tissues studied.

2. Chelate concentration had no effect on the deposition of yttrium in any of the tissues studied.

3. In both dogs and guinea pigs administration of ionic yttrium resulted in concentration largely in the spleen and liver.

4. In dogs EDTA and HEDTA chelated with yttrium with added carrier produced the highest concentration of yttrium in the gastric mucosa.

5. In guinea pigs HEDTA chelated with yttrium with added carrier produced significant amounts in the gastric wall.

6. When carrier free yttrium^{90} was used none of the chelates tested produced a high concentration in the gastric mucosa.

BIBLIOGRAPHY

1. Code, C.F., & Varco, R.L., Chronic Histamine Action, Proc. Soc. Exp. Biol. & Med. 44:475, 1940.

2. Bruegel, C. Cited by Ricketts, W.E., and Associates. (13)

3. Bensaude, Solomon and Dury. Cited by Case, J. T., and Boldyreff, W. N. (8)

4. Viviani. Cited by Ricketts, W.E., and Associates. (13)

5. Emery, E. S., Peptic Ulcer: Its Treatment By The Roentgen Ray: New Eng. Jl. Med. 206: 717, 1932.

6. Portis, S.A., & Ahrens, R., Effects of Shorter Wave-Length Roentgen Ray on Gastric Secretion of Dogs. Am. J. Roentgenol, 11:272, 1924.

7. Ivy, A.C., McCarthy, J.E., & Orndorff, B.H., Studies on the Effect of Roentgen Rays on Glandular Activity. J.A.M.A. 83: 1977, 1924.

8. Case, J.T., & Boldyreff, W.N., Influence of Roentgen Rays Upon Gastric Secretion. Am. J. Roentgenol, 19:61, 1928.

9. Snell, A.M., & Bollman, J.L., Gastric Secretion Following Irradiation of Exposed Stomach and Upper Abdominal Viscera by Roentgen Rays. Am. J. Dig. Dis. & Nutrition. 1:164, 1934.

10. Levin, E., Palmer, W.L., & Kirsner, J.B., Observations on the Diagnosis, Treatment and Course of Gastric Ulcer: Evaluation of Gastric Radiation as an Adjunct in Medical Treatment. J.A.M.A. 156:1383, 1954.

11. Levin, E., Clayman, C.B., Palmer, W.L., & Kirsner, J.B., Observations on the Value of Gastric Radiation Therapy in the Treatment of Duodenal Ulcer. Gastroenterology 32:42, 1957.

12. Ricketts, W.E., Palmer, W.L., Kirsner, J.B., and Hemann, A., Radiation Therapy in Peptic Ulcer: Analysis of Results. Gastroenterology 11:789, 1948.

13. Ricketts, W.E., Kirsner, J.B., Humphreys, E.M., and Palmer, W.L. Effect of Roentgen Radiation on Gastric Mucosa. Gastroenterology 11:818, 1948.

14. Jenkins, J.A., AND McGeorge, M., Control by Radium for Gastric Acidity, Arch. Int. Med., 70:714, 1942.

15. Simon, N., Suppression of Gastric Acidity With Beta Particles of P^{32}. Science 109:563, 1949.

16. Douglas, D.M., Ghent, W.R., & Rowlands, S.; Production of Hypochlorhydria by Beta Irradiation of the Stomach, Lancet 1:492, 1951.

17. Brown, C.H., Therapeutic Principles in Management of Peptic Ulcer. Amer. J. Dig. Dis. 4:1066, 1959.

18. Littman, A., Fox, B.W., and Teplitz, R., Intragastric Beta Radiation Therapy With Radioactive Ruthenium-Rhodium^{106} in Patients With Duodenal Ulcer. Unpublished.

19. Dudley, H.C., Influence of Chelates on the Metabolism of Radioyttrium (Y^{90}). J. Lab. & Clin. Med. 45:792, 1955.

20. Oak Ridge National Laboratory, Oak Ridge, Tennessee, Personal Communication.

21. Hamilton, J.G., The Metabolic Properties of the Fission Products and Actinide Elements. Rev. Mod. Phys. 20:718, 1948.

22. Dobson, E.L., et al. Studies with Colloids Containing Radioisotopes of Yttrium, Zirconium, Columbian and Lanthanum, II. J. Lab. & Clin. Med. 34:305, 1949.

23. Schubert, J. Effect of Zirconium and Sodium Citrate on the Metabolism of Plutonium and Radioyttrium. J. Lab. & Clin.Med. 34:313, 1949.

24. Kidman, B., Tutt, M.L., and Vaughan, J.M. Retention and Excretion of Radioactive Strontium and Yttrium (Sr^{89}, Sr^{90} and Y^{90}). J. Path. & Bact. 62:209, 1950; 63:253, 1951; Nature 167:858, 1951.

25. Walker, L.A. Localization of Radioactive Colloids in Lymph Nodes. J. Lab. & Clin. Med. 36:440, 1950.

26. Rayner, B., Tutt, M., and Vaughan, J. The Deposition of Y^{90} in Rabbit Bones. J. Exper. Path. 34:138, 1953.

27. Cohn, S.H., Gong, J.K., and Fishler, M.C. Studies of EDTA Treatment of Internal Radioactive Contamination. Nucleonics 11:56, 1953.

28. Lewin, Ruth, et al. Distribution of Y^{90} in Ascites Tumor Mice. Science 119:880, 1954.

29. Dudley, H.C., and Greenberg, J. Influence of Chelates on the Metabolism of Radioyttrium (Y^{90}). II. J.Lab.& Clin. Med. 47:891, 1956.

30. Dudley, H.C., and Greenberg, J. Influence of Chelates on the Metabolism of Radioyttrium (Y^{90}). III. J.Lab. & Clin. Med. 52:533, 1958.

31. Harrison, R.C., Margolus, B.D., and Morrison, R.T., Unpublished.